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Biomarkers of the tobacco-specific N-nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)

**Anthony R. Tricker, Ph.D.
Philip Morris Europe**

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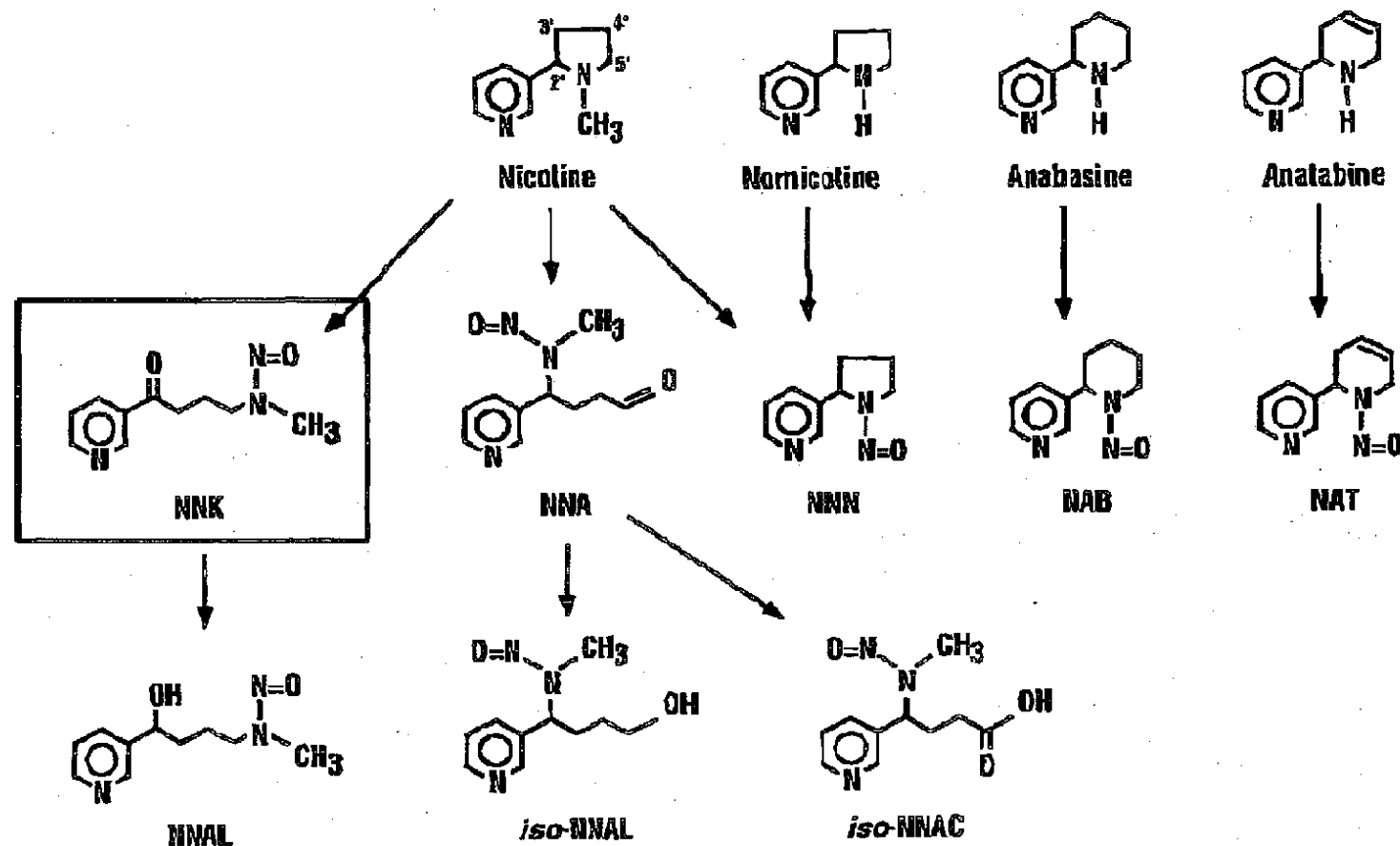
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In vitro formation of TSNA



Hecht and Tricker (1999). Nitrosamines derived from nicotine and other tobacco alkaloids. In: JW Gorrod and P Jacob (Eds.) *Analytical determination of nicotine and related compounds and their metabolites*. Elsevier, Amsterdam. pp. 421-488

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International Agency for Research on Cancer

IARC classifications for TSNA:

Group 2B: Possibly carcinogenic to humans

**4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)
N-nitrosoornicotine (NNN)**

Group 3: Not classifiable as to their carcinogenicity to humans

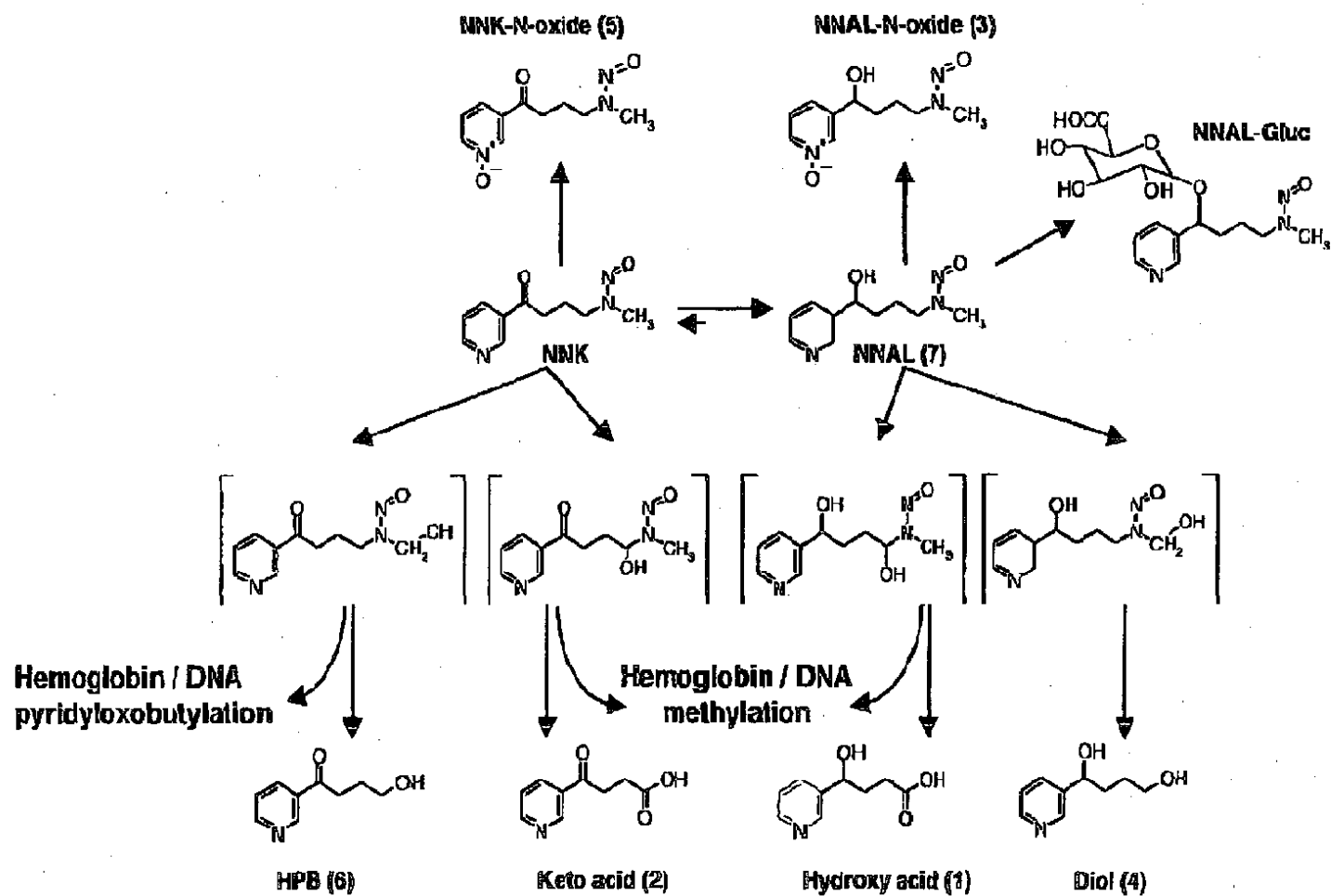
**N-nitrosoanabasine (NAB)
N-nitrosoanatabine (NAT)**

International Agency for Research on Cancer (1985). IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. *Tobacco Habits Other than Smoking; Betel-Quid and Areca-Nut Chewing; and Some Related Nitrosamines*. International Agency for Research on Cancer, Lyon.

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Proposed metabolism of NNK



Hecht and Tricker, 1999 (simplified)

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***In vitro* metabolism of NNK in lung and liver**

Extensive data for rodent tissue metabolism

- 0.6-68 % metabolism to NNAL
- 15-47 % α -hydroxylation to DNA - reactive intermediates
- Pharmacokinetic data for metabolism by α -hydroxylation
- α -hydroxylation correlated with DNA adduct formation

Limited data for human tissue metabolism

- >96.4 % metabolism to NNAL
- 0.9 % α -hydroxylation to DNA - reactive intermediates
- No pharmacokinetic data
- DNA adduct formation requires confirmation

No comparative data for rodent / human metabolism

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Comparative metabolism and adduct formation

Comparative *in vitro* metabolism of [5-³H]NNK, [5-³H]NNAL and [5-³H]NNN and DNA adduct profiles in human, A/J mouse, F344 rat and SG hamster lung and liver under identical experimental conditions

- Precision-cut tissue slices in dynamic organ culture
- Tissues <1 hr old at start of incubation at pH 7.4 and 37°C for 6 hr
- Tissue vitality monitored by LDH leakage and intracellular K⁺ content
- DNA tissue adduct profiles determined by GC/NICI-MS
- Michaelis-Menton pharmacokinetics of all major metabolic pathways calculated over a 2 nM - 100 μM substrate concentration range

Mainstream smoke uptake predicted to result in 2-10 nM NNK and NNN in lung surfactant layer

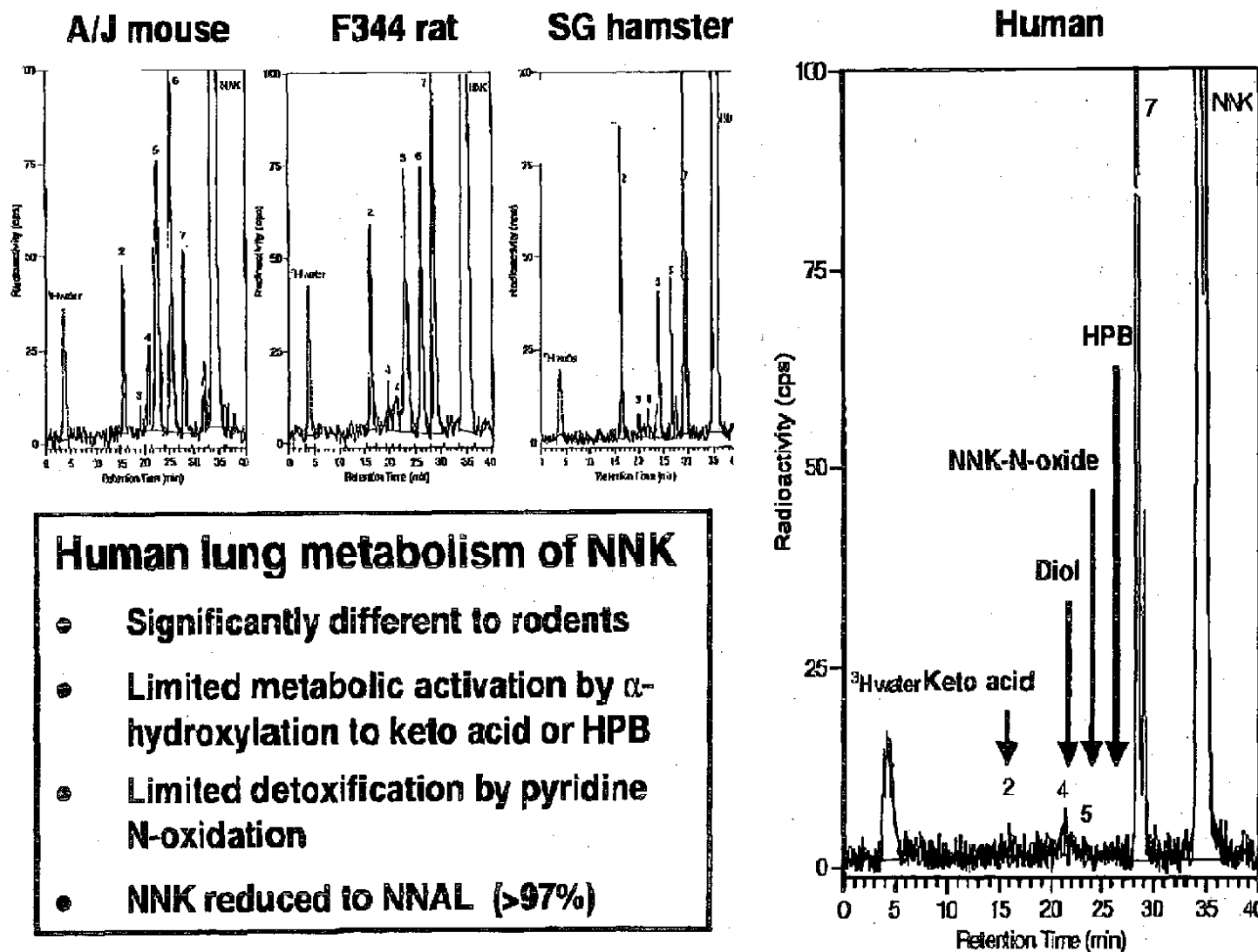
<114 nM NNAL measured in smoker blood plasma

Hecht *et al.*,
Cancer Res., 59:590-596, 1999

Systemic blood TSNA concentrations reach 100 μM in rodents under bioassay protocols

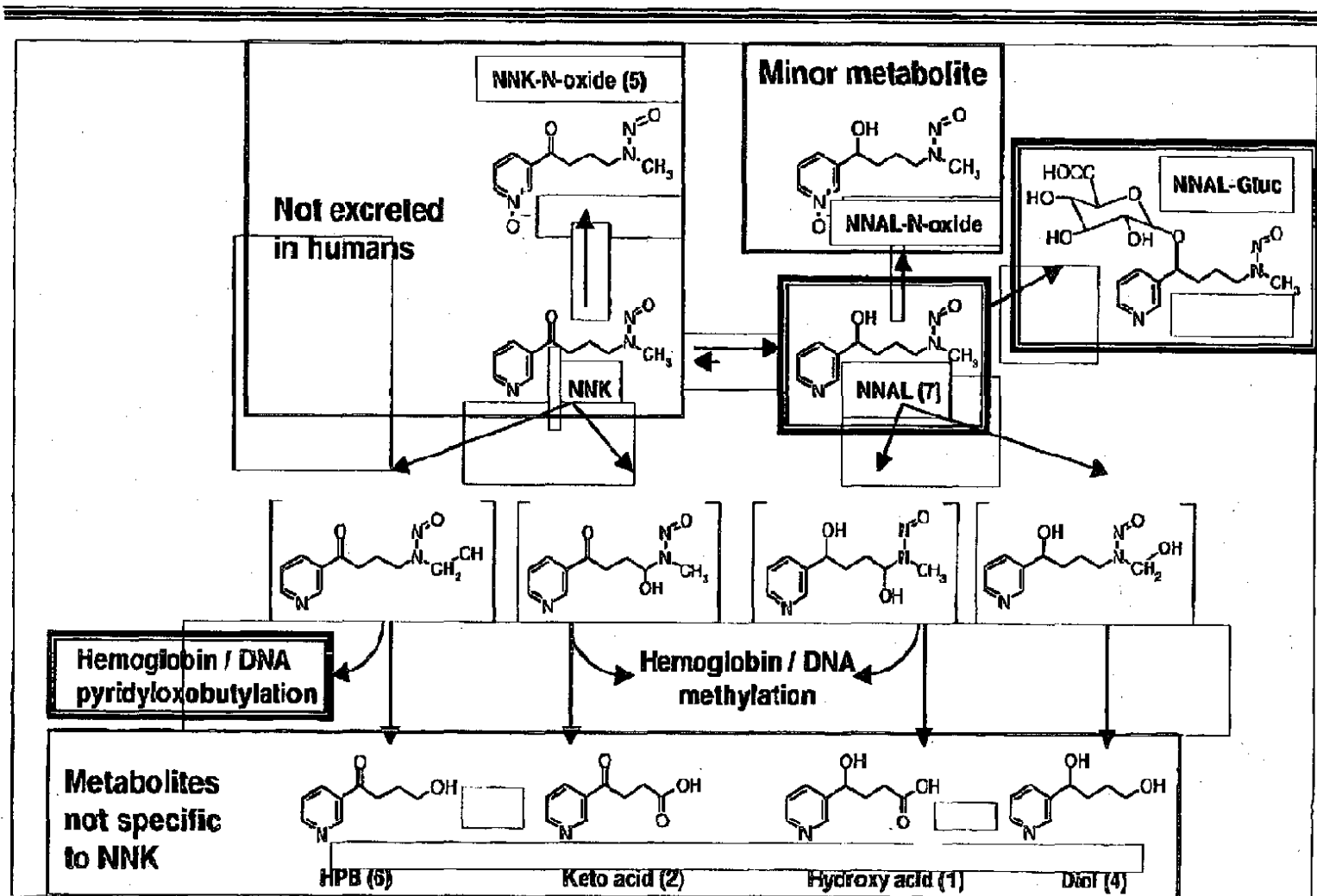
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Metabolism of 0.1 μ M NNK by lung tissues from different species under identical experimental conditions



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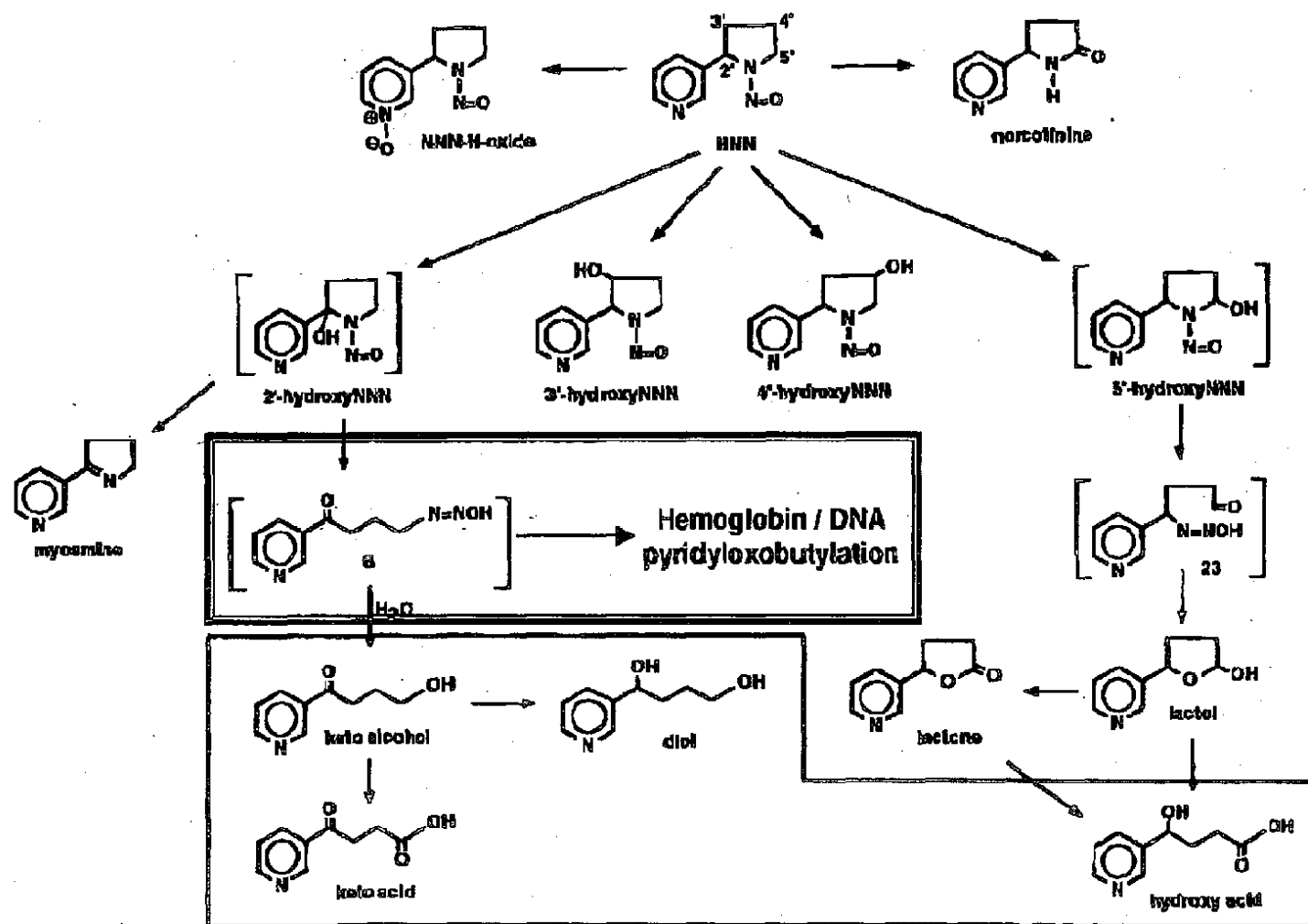
Potential biomarkers of NNK exposure



Hecht and Tricker, 1999 (simplified)

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Proposed metabolism of NNN



Hecht and Tricker, 1999

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Biomarkers of NNK exposure: urinary metabolites

| Smoking status | ETS exposure (hours/week) | Cotinine (mg/day) | NNAL + NNAL-Gluc (pmol/day) | NNK equiv. (µg/day) |
|----------------|---------------------------|-------------------|-----------------------------|---------------------|
| NS | 3.6 ± 6.5 | 0.018 ± 0.013 | < LOD | - |
| NS | 19.8 ± 27.2 | 0.044 ± 0.042 | 43.3 ± 44.4 | 0.009 ± 0.010 |
| S | (20.8 ± 4.6 cpd) | 3.030 ± 1.660 | 9861 ± 43880 | 2.04 ± 0.91 |

Limit of detection (LOD) = 1.5 pmol NNAL + NNAL-Gluc/day
cpd = cigarettes per day

Meger *et al.*, *Biomarkers*, 5:33-45, 2000

" Thus, NNAL and NNAL-Gluc appear to be sensitive biomarkers for NNK uptake. "

Hecht and Tricker, 1999

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NNAL and NNAL-Gluc are specific for NNK uptake

NNK and other TSNA are not formed *in vivo* following

- **Buccal absorption of nicotine (12-40 mg)**

Tricker *et al.*, *Carcinogenesis*, 14:1409-1414, 1993

- **Oral administration of cotinine (40-60 mg)**

Tricker *et al.*, *Carcinogenesis*, 14:1409-1414, 1993

- **Transdermal nicotine administration (15 mg)**

Hecht *et al.*, *Cancer Res.*, 59:590-596, 1999

No endogenous formation of NNK from nicotine

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Biomarkers of NNK exposure: hemoglobin adducts

| HPB-releasing adducts | | Reference |
|------------------------|---------------------------|------------------------------------------------------------------|
| Smokers (fmol/g Hb) | Nonsmokers (fmol/g Hb) | |
| 80 ± 190 | 29 ± 26 | Carmella <i>et al.</i> , <i>Cancer Res.</i> , 50:5438-5445, 1990 |
| 69 ± 44 | 34 ± 16 | Falter <i>et al.</i> , <i>Clin. Invest.</i> , 72:364-371, 1994 |
| 55 ± 46 | 27 ± 35 | Branner <i>et al.</i> , <i>Biomarkers</i> , 3:35-47, 1998 |
| 26 ± 12 | 19 ± 8 | Atawodi <i>et al.</i> , <i>CEBP</i> , 7:817-822, 1998 |

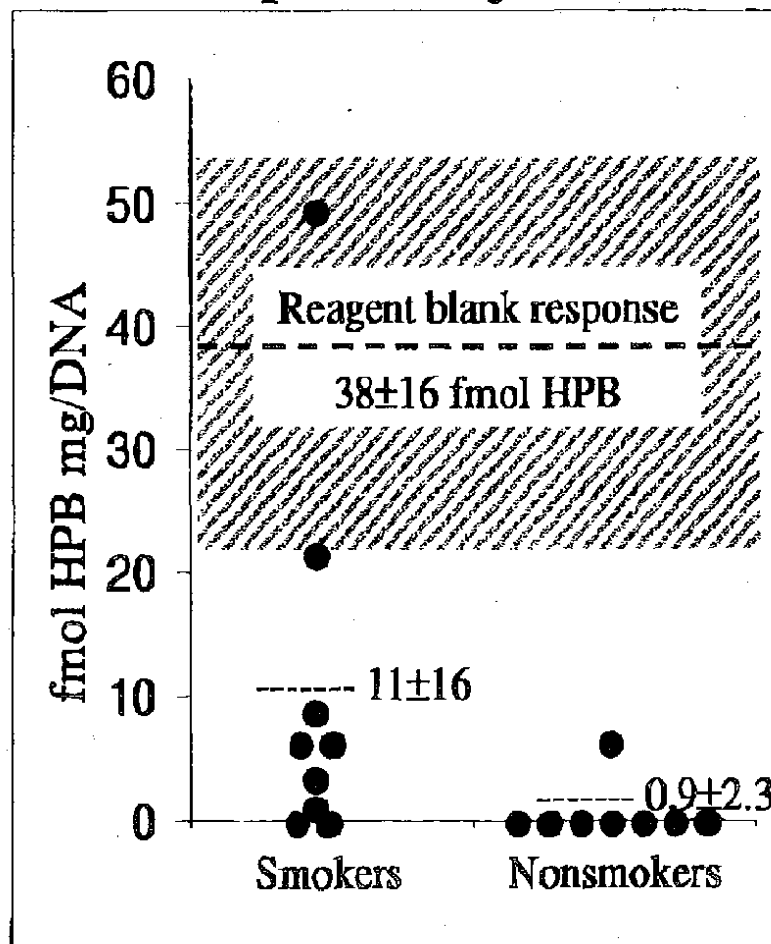
“ Thus, HPB-releasing hemoglobin adducts have limited utility as biomarkers of exposure to TSNA in smokers because adduct levels are frequently not much higher than assay background amounts, and are unsuitable as biomarkers of ETS exposure in non-smokers. “

Hecht and Tricker, 1999

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Biomarkers of NNK effect: lung DNA adducts

AHF pilot study results



AHF pilot study claiming the presence of HPB-releasing DNA adducts in lung of both smokers and nonsmokers

Foiles et al., CRT, 4:364-368, 1991

NCI study of 32 lung autopsy samples from smokers found no detectable HPB-releasing adducts

Blömeke et al., Carcinogenesis 17:741-748, 1996

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Summary

- **Metabolism of NNK, a rodent lung-specific carcinogen, is different in laboratory animals compared to man**
- **Selective and sensitive urinary biomarkers exist for the determination of NNK exposure and uptake in man**
- **No endogenous formation of TSNA from nicotine in man**
- **HPB-releasing hemoglobin adducts of NNK and NNN are not a suitable biomarker of exposure in man**
- **NNK-derived HPB-releasing DNA adducts in human lung are not supported by *in vitro* metabolism studies**

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